VIII. *Onygena equina*, Willd., *a Horn-destroying Fungus.*

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Received April 6,—Read May 5, 1899.

[Plates 21–24.]

Some time ago Mr. Crossland, a student in this laboratory, brought me a cow's horn much decomposed, and covered with a fine growth of a fungus—*Onygena*. The specimen had been found lying on the wet grass on a hill in Derbyshire. The decomposing horn evolved a strong odour of ammonia, and its outer parts flaked off easily as cheesy, skin-like, or almost papery, opaque, white bits, the handling of which left a penetrating ammoniacal odour on the fingers, reminding one of the smell of peptone which has been kept in too damp a place. A glass rod dipped in HCl, and held near the horn, was at once surrounded by a white cloud. Underlying the ammoniacal odour, however, there seemed to me to be another and more persistent sour smell.

Since very little is known of this fungus, I have made a thorough examination of it.

The remarkable genus *Onygena* comprises some half-dozen species of fungi of peculiar habitat, since they all* grow on animal bodies, such as hoofs, horns, feathers, hairs, and skins, and thus present characteristics shared by very few other true fungi, *e.g.*, the Laboulbeniaceæ, and the genus *Cordyceps* among the Hypocreaceæ, both of which grow on insects. Berkeley† found one species of *Onygena* on a piece of old flannel in Sherwood Forest, and remarks, "which had probably formed part of the dress of some gipsy." The little that is known of certain epidermal parasites on human hair and skin, shows they are quite different fungi.

The older mycologists entertained various ideas as to the systematic position of the genus *Onygena*. Persoon‡ placed it with the Myxomycetes *Licea* and *Tubulina*, evidently laying stress on the loose powdery spores, though he failed to recognise the capillitium-like threads in the fructifications; but since he placed these fungi between *Mucor* and *Æcidium*, we need hardly enter further into the discussion of his views.

* The *Onygena faginea* of Fries was *Pilacre faginea* (B. and Br.) and not an Ascomycete at all.

† 'Introd. to Crypt. Bot.,' p. 272, 1857.

‡ 'Syn. Fung.,' pp. 15 and 203.

WITHERING* and others corrected PERSOON's error in denying the existence of barren filaments (*capillitium*) between the spores of the ripe fructification, but none of these authors really understood the true nature of these filaments. ALBERTINI and SCHWEINITZ,† who seem to have quite apprehended the weakness of the alliance between *Onygena* and the Myxomycetes, made some advance in placing the former near *Lycoperdon* and *Tulostoma*, and although BOLTON, WILLDENOW, WITHERING, and subsequently PERSOON himself accepted similar views, while FRIES‡ varied the systematic position by associating *Onygena* with *Asterophora*, *Tulostoma*, *Lycoperdon*, and *Polysaccum* into one group, the genus was by no means understood. In 1844 § the TULASNES showed that the spores are developed in asci "*à la manière des spores des Tubérées des Erysiphe*," and pointed out that the whole nature of the fructification differs from that of the Myxomycetes and of the Lycoperdineæ, and this was the first time that any approach to a real comprehension of the systematic position of the genus was possible. But although the TULASNES brought *Onygena* ‖ into its proper group—the Ascomycetes—very little progress has been made since, and the bibliography is correspondingly scanty.

Even DE BARY in 1884 was unable to add much to these results. He points out¶ that the early stages of development of the fructification are too little known to enable us to trace their homologies, and they can only be called "sporocarps" in the sense employed for the rest of the Ascomycetes, because they contain asci, and present certain resemblances to the fructifications of these fungi. His account of the structure** is essentially that of TULASNE. From the mycelium arise a number of small solid columns, each surmounted by a globoid receptacle full of spores; these are the pedicellate sporocarps. The spore-case proper (*peridium*) contains a brown powdery mass of spores and intermingled hyphæ (*capillitium*), and was known as the *gleba*. All these terms—*peridium*, *gleba*, *capillitium*, &c.—derived in the first place from supposed analogies with Gasteromycetes, have as a rule been upheld by subsequent writers.

Nothing was known definitely as to the germination of the spores or the early stages in any species of *Onygena* when I began this work. Moreover, very little information about this fungus can be obtained from the usual sources. The name of the genus does not occur in VON TAVEL;†† it only occurs in two notes of one line each in ZOPF,‡‡ one referring to the fructification of *Onygena* as an example of a negatively geotropic organ, and the other mentioning the curious habitat of the fungus.

* 'Bot. Arrang. of Brit. Plants,' 3rd ed., vol. iv., p. 378.

† 'Conspect. Fung. Agri. Nisk.,' p. 113.

‡ 'Syst. Mycol.,' p. 51.

§ 'Ann. des Sc. Nat.,' Ser. III., Bot., vol. 1, pp. 367–372. With plate and literature.

‖ They were unable to study living specimens of *O. equina*, *loc. cit.*, p. 370.

¶ 'Morph. and Biol. of Fungi,' Engl. ed., p. 193.

** *Loc. cit.*, p. 196.

†† 'Vergl. Morph. d. Pilze,' 1892.

‡‡ 'Die Pilze,' 1890, pp. 208 and 227.

LUDWIG,* to whom I often turn for curious notes on biology, does not even name the genus. Nor is it mentioned in MASSEE'S 'British Fungus Flora,'† although he gives the Gymnoasceæ, near which Onygenei are usually placed.

All this goes to show that our knowledge of this genus of fungi is meagre and incomplete, and points to the desirability of its exact study. I have also been able to discover very few references to the physiology of the genus *Onygena*. ZOPF‡ says the sporangiophores of *Onygena corvina* are negatively geotropic, but the remark seems to imply no more than the simple observation that they stand erect on the substratum.

But DE BARY adds a significant observation§ which may be taken regarding the conditions of germination in *O. corvina*. The spores refused to grow in water or in nutrient solutions, or in gastric juice, at any temperatures, "but there was a fine formation of compound sporophores of *Onygena* on the hairs cast up by a white owl . . . . . which had received the spores of the fungus with a mouse which it had eaten; the fungus developed on the hair from the mouse on which the spores had been strewed and from no other." As will be shown later, I have had better success in this connection.

The most recent work on the systematic position of *Onygena*, that of FISCHER,‖ places it among the Ascomycetes in the Order Plectascineæ, together with the Gymnoasceæ, Aspergilleæ, Elaphomyceteæ, &c.

The habitat and habit, the thin peridium and its peculiar dehiscence, the irregularly scattered and jointed asci with eight spores, which are one-celled, and the absence of conidia, are the principal group characters.

The genus characters are given as follows:—

Fructification rounded, usually nearly globular, stalked or sessile, and about 1–5 millims. in diameter; clothed with a pseudoparenchymatous or membranous *peridium*, dehiscing at maturity by an annular rupture, or breaking up irregularly or into lobes. The internal mass (*gleba* of earlier writers) composed of filamentous hyphæ (*capillitium* of earlier writers) and densely crowded *asci*. Asci more or less irregularly globoid, 8-spored. Spores 1-celled, smooth or sculptured, lying without order in the ascus, and filling the peridium, when ripe, as a powdery and mostly brown mass, often intermixed with *capillitium fibres*.

The six species known occur in Middle and North Europe, Italy, France, and N. America, and are distributed into two sub-groups, four of them having the typical stalked fructification and smooth spores, forming the first group, while *O. caprina*

* 'Lehrbuch der niederen Kryptogamen,' 1892.

† London, 1895.

‡ 'Die Pilze,' Breslau, 1890, p. 208.

§ *Loc. cit.*, p. 351.

‖ ENGLER and PRANTL, 'Pflanzenfamilien,' Th. i., Abth. 1, p. 309.

(FUCKEL) and *O. mutata* (QUÉLET) have sculptured spores and sessile fructifications, and approach the Aspergillaceæ.

Of the typical species, *O. equina* (WILLD.) Pers. has the fructification globular or depressed, with the pedicel approximately of equal thickness throughout. Peridium whitish, or pale to reddish-brown. Spore-mass with well-marked capillitium. Asci 14–22 μ long by 10–14 μ broad; spores 7–9 × 4–5 μ, yellowish. It occurs on decomposing hoofs and horns of cattle, sheep, horses, &c.

O. corvina (ALB. and SCHW.), including *O. Piligena* (FR.), has longer stalks tapering above, and hardly any trace of capillitium; smaller asci (8–10 × 7–8 μ) and spores (5–8 × 2–3 μ), and occurs on feathers, fur, and wool.

O. arietina (FISCHER) has a hollow stalk, an annular furrow at the base of the peridium, and was found at Davos on the horn of a living ram; since the specimen was too old to discover the asci, this species is doubtful.

There can be no doubt as to the identity of my specimens with *O. equina*, and I now proceed to the results of my own investigation.

In describing the anatomy and histology of the fungus, it will be convenient to refer to the following parts:—(1) the vegetative mycelium running on and in the horn; (2) the pedicel of the fructification; (3) the globoid sporocarp, consisting of the "*peridium*" and the "*gleba*," as we may still term them for purposes of description; and (4) the spores.

The material for detailed work was hardened in Rath's solution* for a few hours, and passed through the dilute alcohols to absolute alcohol; then placed successively in xylol-alcohol, xylol, xylol-paraffin, and finally into blocks of paraffin of M.P. = 60° C. The sections were cut with a microtome and mounted as usual.

In other cases dilute Fleming's solution or Keiser's mercuric chloride solution were employed:

Various stains were used, but it was not always necessary to stain at all, since the dark colour caused by the osmic acid of the Rath's solution often suffices to pick out details, except in special cases.

For certain purposes—for instance, when dissecting out the ascigerous branches—I found it better to isolate the sections, and swell them in Eau de Javelle, or dilute potassium hydrate and mount in glycerine. The vegetative mycelium is composed of copiously-branched and septate hyphæ, which have rounded tips and are about 3–5 μ in thickness. As observed fresh, and growing in one of the food-media mentioned on pp. 276 and 277, the vigorous hyphæ appear homogeneous and glistening, especially at the tips, and the septa are hardly discernible; but in preserved mycelia each hypha is found to have firm cell-walls and septa, and contents which vary considerably in quantity and degrees of granularity.

Sections cut in the plane of the scaly structure of the cow's horn—*i.e.*, tangential

* See OLTMANN, 'Bot. Zeit.,' 1898, h. 6–8, p. 100; and ZIMMERMANN, 'Zellkern,' p. 3.

to the surface—show the mycelium as a dense plexus of branched and anastomosing hyphæ, with numerous septa and large drops or granules deeply stained by osmic acid in preparations from Rath's fluid (figs. 11–13).

No signs of cellulose reaction could be obtained with the cell-walls, and there can be little or no doubt that *chitin* is the principal constituent of the hyphal membranes, though the conclusive reactions for this were obtained from aërial parts, as will be shown later.

No "clamp-connections" or other characteristic marks could be discovered on the vegetative hyphæ, nor were any pits observed in the septa. Definite nuclei appear to be absent from these hyphæ, their only representatives being certain extremely minute stainable granules.

The drier parts of the fully or nearly decomposed horn present a remarkable appearance of laminated, thin, pure white films, which separate like plates of mica into finer and finer sheets like paper. The fresh films are marked with delicate vertical straight lines, like fluting, and under the microscope they are seen to be areolated into yet smaller and thinner plates, finely punctate, and probably representing the ultimate plates of horn.

Hot water has no appreciable effect on this papery substance, but it swells up in potassium hydrate, without dissolving. Dilute sulphuric acid brings about a rapid swelling and complete solution, especially on heating, the swelling pieces turning yellow; the solution is perfectly clear, and rapidly turns purplish-pink to brown-pink.

Acetic acid, even hot, only causes very slight swelling; ammonia hardly affects it. Nitric acid causes rapid swelling and disintegration into smaller scales, which then swell further and dissolve, turning canary-yellow as they do so. Ammonia causes this yellow to deepen in hue.

Hydrochloric acid, diluted to 50 per cent., does not swell or dissolve the scales, even on heating.

The papery films stain deeply in all ordinary dyes—fuchsin, methylene blue, gentian violet, &c.—and retain them tenaciously. Microscopic examination shows the fungus hyphæ running all over between even the thinnest scales, and the outlines of areolæ, which I think must be the ultimate scales, are visible.

I made many attempts to bring out any evidence of the presence of bacteria in these films, but it seems certain that none are present in the areas occupied by the hyphæ, and it must be concluded that the mycelium does its work of disintegration unaided by bacteria.

It is true I found rodlets once or twice here and there in the badly decomposed cheesy parts at the edges of the destroyed horn, but there was no constant relation between their presence and the degree of destruction, nor could I trace any connection between their appearance and that of the fungus. No doubt was left in my mind that what bacteria do occur, follow on the ravages of the fungus, and quite accidentally. Moreover I was unable to get any definite signs of activity on sterilised

horn shavings and plates of these bacteria. It is, however, to be noted that there is a decomposition of horn—I know it in connection with the decomposition of hoofs—in which bacteria alone appear to be concerned, but that raises a different question which I do not propose to consider here. The point for the moment is that no symbiosis between any bacterium and the *Onygena* exists; the fungus carries on its destruction alone, and I see no reason for doubting that this fungus may alone be sufficient to account for the ammoniacal decomposition of hair, horn, and hoof shavings employed for manure in the hop fields and elsewhere. Obviously, also, the results have their bearings on questions of the destruction of human hair, skin, &c., under the action of epidermal parasites.

The question as to the constitution of the cell-walls of the hyphæ was attacked in connection with the sporophores, and especially the pedicels, because the mycelium in the horn might give equivocal results owing to the presence of keratin and its derivatives, or other bodies which would affect the reactions. Vertical sections of the sporophores were placed in sealed tubes in 80 per cent. potassium hydrate, and heated to 160° C. for two hours; on removing these sections to 90 per cent. alcohol, even the thinnest remained well together—they must not be put into water, as they then swell up and disintegrate. On now testing with iodine solution and dilute sulphuric acid the characteristic plum-pink reaction of chitin* was very marked in hyphæ and spores alike.

The mature sporophore (Plate 21, figs. 1–4) is shaped like a miniature "button mushroom," or a drumstick with a very short and relatively stout handle. The dimensions vary considerably, but are usually between the following:—

Total height of sporophore	5–8 millims.
Diameter of head	2–4 ,,
Length of pedicel	4–6 ,,
Thickness of pedicel	1–2 ,,

Vertical sections show the pedicel to be a compact and solid mass of interwoven hyphæ, with a mainly upward course, and showing no special differentiations into thick and thin, inner and outer, or empty and full hyphæ. They appear to be all alike (figs. 5, 6, and 10). Just below the head, however, the hyphæ are compacted into a very dense layer (figs. 3, 4, and 10), which stretches horizontally and forms a sort of floor to the spore-case (head), and reminds one of a sort of hymenium, or a sub-hymenial layer. This compact floor is often arched up slightly into the spore-case, and is evidently the *columella* referred to by FISCHER;† it has no significance, I believe, in any such connection, but it is continuous at the margins into the *peridium* (fig. 4). The latter (figs. 7 and 8) is a compact pseudoparenchyma, ending

* See WISSELINGH, in 'Pringsh. Jahrb. f. Wiss. Bot.,' vol. 189, p. 609.

† FISCHER, *loc. cit.*, p. 103.

externally in ragged remnants of cell-walls, and passing internally into the ordinary hyphæ of the "capillitium." The further inter-relations of these parts will be made clearer when I have described the development.

When the fresh ripe sporophore is cut vertically, the yellowish-brown spores (gleba) are seen filling up the cavity between the peridium and the pedicel as a compact dark mass, which at first appears homogeneous (fig. 2*c*). Closer examination shows that the spores are entangled in irregular meshes between a system of hyphal strands—now practically empty—which run principally, but not only, upwards and downwards from the floor to the roof of the spore-cavity, and are linked by cross-connections in various ways (figs. 8–10). These hyphal strands constitute the "capillitium," and if we wash out the spores from the interstices between them, they are found to form a pretty regular system of branching strands running vertically from the floor, and branching in a fountain-like manner (but with numerous cross-connections) till they end in the peripheral peridium, the compacted cells of which are, indeed, merely their swollen and fused ends which have undergone cell-divisions in all planes (figs. 7 and 8).

At maturity the peridium bursts, either irregularly or by separating in a more or less circum-scissile manner from the junction below with the floor of the spore-case (fig. 2*c*). The spores, lying quite loose as a dry powder in the meshes of the now withering capillitium, are slowly scattered, and hitherto no one has explained their further fate or behaviour.

Most of the above bare facts of the coarser anatomy, &c., are known, but it was necessary to verify them, as I have done, in order to make clear what follows.

A point of great interest concerns the asci. It can afford no surprise that these should have been so long overlooked. As I have shown, the ripe spores lie quite loose, as a powder, in the meshes of the capillitium (fig. 10), and even careful examination may fail to reveal an ascus in the ripe sporocarp. Indeed, for a long time I was driven to suspect that, after all, the spores are developed in some other way, and only a thorough investigation of the development of the sporocarps and spores led to success in clearing up this difficult point.

Before proceeding to describe the development, however, I propose to give the results of my cultures of these spores. As already stated, no one had as yet succeeded in persuading the spores to germinate. On the basis of the foregoing knowledge I proceeded with attempts to germinate the spores, easily obtained in abundance and quite ripe.

A sporocarp was crushed by a sterile needle in water, and the yellow-brown "gleba" rubbed and shaken until a drop of the water showed the spores were well distributed. A tube of yeast-extract, saccharose, and gelatine was then thoroughly infected, and hanging drop cultures made as usual.* No traces of germination could be obtained in this medium, however, though I usually find it a very satisfactory one

* See MARSHALL WARD, 'Phil. Trans.' B., 1892, p. 130, for details of methods.

for fungi. Bearing in mind De Bary's remarks, quoted above, and the following observation of Eidam's, I tried this medium neutral, acidified with acetic acid, and rendered alkaline with ammonia, but without success. Eidam* found the germination of the spores of *Gymnoascus* was promoted by the addition of acetic acid, but not by ammonia. This fact is significant, because the allied genus *Ctenomyces* is found growing on feathers, though its spores germinate readily in dung. The medium referred to was chosen because it was at hand, and I often find it useful, as said.

Having regard to the natural substratum, I next tried gelatine and glue made up in various ways without sugar, arguing from the fact that glue-like bodies are obtainable from hoofs, skins, horn, &c. The first trials were made with glue and tap-water; glue, gelatine and tap-water; glue and various mineral solutions; gelatine and mineral solutions; but in no case could I get the spores to germinate either at ordinary temperatures or at 25° and 35° C. in the incubator. After many failures the idea occurred to try whether attempts with artificial gastric juice could be made to succeed, for although De Bary had failed with extract of pig's stomach, his note on the growth of *Onygena* on the owl's cast suggests that some connection between digestion and germination of these obstinate spores may, nevertheless, exist. The results proved that this is the case.

A solution was made as follows:—200 milligs. of pure artificially prepared pepsin were dissolved in 100 cub. centims. of water, and 100 cub. centims. of a 0·4 per cent. solution of hydrochloric acid added.

Spores were then sown—in tubes and in hanging drops—in this raw gastric juice; in the same diluted; and in the same to which various decoctions of glue, gelatine, and other presumed food-materials were added.

Further trials were made, in which either the spores alone, or the food medium alone, were exposed to the action of the gastric juice.

Moreover, the experiments were varied as to temperature, dilution, length of digestion, and other treatment of the spores.

It soon became evident that the gastric juice *is* an efficient agent, and De Bary's failure with extract of pig's stomach must be put down to some accessory circumstance.

The first success was with spores shaken up in water, to which the gastric juice was then added. In hanging drops at 23° C., some of the spores were found to be putting forth germ tubes on the fourth day, and the process was well advanced on the fifth day (fig. 36), but no further progress was made. Similar success attended sowings in glue-solution† to which gastric juice was added, the treatment in other respects being exactly the same.

Similar cultures at 36° C. gave negative results. In both these series there are reasons for believing that the prolonged action of the gastric juice eventually stopped

* Cohn's 'Beiträge,' vol. 3, 1880, p. 296.

† Made up as follows:—Glue, 10 grams; tap water, 500 cub. centims.; Liebig's meat extract, 1 gram; peptone, 1 gram.

the growth, since I had added a large proportion—about 50 per cent.—to the cultures. Further experience also suggests that in the first series the partially digested remains of some of the spores served the others as food for a time. In the second series it is probable that the products of digestion of the glue and Liebig's extract inhibited the further development. No success attended sowing of spores digested for four hours at 35° C. in the gastric juice, and then sown in bouillon-pasteur, or levulose yeast-extract; but this may have been in part owing to the rapidity with which these liquids turned foul owing to introduced bacteria and yeasts, &c., with the spores from the horn. Spores kept for twenty-four hours in gastric juice at 36° C. and then dried on sterile cover-slips for twenty-four hours, failed to germinate in glue-gelatine broth—both with and without the further addition of yeast-extract-levulose, or of gastric juice—at 20°–25° C., whence may no doubt be inferred that the digestion was carried too far, as I found similar failures occurred later, in far better food-media, when the spores had received such drastic exposure to the action of the gastric juice.

Since the mycelial growths obtained in the above experiments were soon arrested, the idea occurred to try the effect of freezing the spores, in order to see whether more vigorous germination could be obtained. The idea was suggested by ERIKSSON'S success with the artificial freezing of the spores of *Uredineæ*.*

Fresh heads of the *Onygena*, containing ripe spores, were exposed from January 27 to February 3 in a glass vessel outside, and experienced a fairly sharp frost during the first night.

The following experiments with these frozen spores are instructive. In mixed glue-gelatine-broth and horn-extract some of the frozen spores, previously soaked for two hours in water only at 35° C., germinated in three days, but got no further than the emission of germ-tubes some two or three times the length of the spore. When the frozen spores were digested for two hours at 35° C. in the artificial gastric juice, however, they germinated much more strongly in the glue-gelatine-broth alone in the same period; the cultures being side by side and absolutely alike in other respects.

It seems noteworthy that this was the only experiment in which I obtained positive results with the ascospores without the use of gastric juice. At the time I thought it showed that freezing caused some change in the spores—perhaps the secretion of pepsin—which enables them to dispense with the gastric juice so far as preliminary germination is concerned; but another explanation seems possible. In later experiments, when I had become familiar with the second form of spores—*chlamydospores*—to be described below, it occurred not unfrequently that in sowings containing both kinds of spore, the chlamydospores germinated out under conditions unfavourable to the ascospores. Since there was some danger of confounding the two kinds when germinated, until I had become more familiar with their peculiarities, it is just possible that in the case described I had done this, and that the successful germination without gastric juice was really that of the chlamydospores. Unfortunately the point must remain obscure for the present.

* ERIKSSON, 'Centralbl. f. Bact.,' *Abt.* 2, 1895, vol. 1, Nos. 15, 16.

Since the glue I was using* did not appear to give very strong mycelia, I tried to extract a solution from the horn itself, to see if something still nearer the natural food of the fungus could be obtained. Various methods were employed, *e.g.*, simply boiling the horn shavings in water, extraction in an autoclave, and, subsequently, a solution of the horn in boiling dilute sulphuric acid neutralised with barium carbonate. I also obtained from a glue factory the first coarse glue extracted from the hoofs, horn, &c., as well as some of the crude materials—mixed skin, hoof, &c.—from which the glue is prepared.

The best results were obtained with the raw coarse glue referred to above. In this medium, to which a few drops of gastric juice were added, the spores germinated freely and strongly in two days at 22° C., and in tubes I was able to obtain large mycelia in the course of a few days. So far it appears to be the best medium for the culture of the spores (fig. 33).

Talking over the question of the constitution of horn, of which very little appears to be known, Dr. RUHEMANN suggested glucose-amine, a body obtainable from *chitin*, as a possibly suitable food-stuff for such a fungus, although glucose-amine is not got from horn. I obtained some pure glucose-amine from GRÜBLER and made it up in various ways. Positive results were obtained when it was mixed with gelatine and mineral solution, provided the spores were added in gastric juice. But the germination was the slowest of any, and did not occur until the 8th to 10th, or even the 14th day, and often ran an abnormal course. I could only infer that it had an inhibiting effect rather than a stimulating one—the positive result being probably due to the products of digestion of the gelatine (see fig. 35).

Having obtained satisfactory evidence that the spores are rendered capable of germination by the action of gastric juice, and that they therefore no doubt pass through the body of the cow in nature, it was obviously desirable to test the effect of an extract of cow-dung on the growth of the mycelium.

Filtered and boiled watery extract of the dung added to gelatine proved a very good medium, and strong mycelia were obtained in the hanging drops, but again, only if gastric juice was also used. Neither freezing nor soaking the spores in water brought about the germination in the extract alone, or with gelatine, or with horn-extract or glue. The slow progress of the specimen figured (fig. 34), is due to the low temperature, 16–18° C., at which the germination occurred in this case. The much better growth at 23° C. in this medium is sufficiently clear from fig. 37, which shows the mycelium on the 7th day.

In all these germinations the gradual disappearance of the oily drops, presumably food supply, are observable from day to day (see figs. 33, 34).

The foregoing experiments seem to establish the following facts :—

(1.) The ascospores are incapable of germination in any of the media employed without previous treatment.

* It was a "fine" glue, and I am told that these pale-coloured glues are treated with antiseptics and otherwise.

(2.) Their germination is promoted by treatment with gastric juice, either by mixing the latter with the food materials, or, even better, by subjecting the spores to a preliminary digestion for 2–4 hours at 35° C.

(3.) In one doubtful case the spores appeared to germinate, without gastric juice, after a preliminary freezing.

(4.) The optimum conditions of temperature are near 22° C., though germination occurs at 16–18° C. and up to 25° C. In no case could I get germination at 34–36° C.

(5.) Too long a digestion is fatal to the germination.

(6.) There are some still unexplored problems regarding the nature of the food materials required. Coarse, crude glue is the best so far; cow-dung and glue or gelatine are also good. Glucose-amine is not a favourable medium. Ordinary carbohydrates, so necessary for most higher fungi, appear to be unnecessary for *Onygena*.

It remains to say a few words regarding negative results in media which one would have expected to be suitable.

In several cases where I used horn-extract, glue, gelatine, &c., even with gastric juice, the hanging drops dried up too much, and the concentrated medium deposited crystals of various sorts. That this over-concentration retards germination was proved by the behaviour in parallel cultures, where condensation of moisture on the cover-slips promoted the swelling and dilution of the drops, and germination then proceeded normally. This occurred several times, and I have confidence in the explanation, which, moreover, is in accordance with the fact that in nature the fungus can hardly obtain other than very dilute solutions from the horn.

The methods employed in studying the rate of growth were the same as I have previously described when measuring the rates of growth of *Bacillus ramosus*,* and the reader may be referred to that description for details. The following may serve as an example, showing the rate of growth of a germinal tube and hypha developed from the spore:—

A spore sown on March 24 at 21° C. in raw glue and gastric juice, had begun to germinate on the 26th. The following table gives the measurements:—

SPORE sown March 24 at 21° C.

Date.	Length of Hypha.	Temperature.	Remarks.
	μ	° C.	
March 26	7	21	
„ 27	22	18	
„ 28	46	18	
„ 29	95	20	One small branch
„ 30	660	21	Abundantly branched

Here we find an astonishingly rapid growth set in on the sixth day; the drop was now large and quite liquid, and the temperature was slowly rising to near the

* See MARSHALL WARD, 'Proc. Roy. Soc.,' vol. 58, p. 24.

optimum. Nevertheless, the mycelium was now much branched, and the energy of growth was being directed into the side branches.

The measurements up to March 29 are accurate, and made under a high power; those of March 30th are only approximate, as they had to be made under a B objective, and each division of the micrometer covered 11 μ, too large a unit for accuracy when the hyphæ are so long.

The irregularities in growth—the measurements only concern the four main hyphæ referred to—are no doubt due in part to the branching, since I have often found that the forward growth of a main hypha is arrested or slowed when the energy of growth begins to affect side branches, and the hyphæ *a* and *b* had formed numerous such branches on March 30.

The following gives a typical example of the rate of growth of the chlamydospore at ordinary temperatures. A pair of chlamydospores germinated together in coarse boiled glue and gastric juice at 21° C. In 48 hours each had put out a germ-tube, which I will term *a* and *b* : $a = 33\ \mu$ and $b = 16{\cdot}5\ \mu$ long. During the next 24 hours

CHLAMYDOSPORES sown March 24 at 21° C.

Date.	Hypha *a*.	Hypha *b*.	Hypha *c*.	Hypha *d*.	Temperature.
	μ	μ	μ	μ	° C.
March 26 . .	33	16·5	...	...	21
„ 27 . .	64·5	48	18·5	17	18
„ 28 . .	138	122	26	49·5	18
„ 29 . .	528	396	158	204	20
„ 30 . .	880	1000	550	440	21

each had put forth another germ-tube, which may be termed *c* and *d*, $c = 18{\cdot}5\ \mu$ and $d = 17\ \mu$; meanwhile, *a* had grown to $64{\cdot}5\ \mu$ and *b* to $48\ \mu$, and this although the culture was now at 18° C.

The further behaviour of these tubes is best shown in the accompanying table.

The following is a good example of the growth of one of the lateral branches on a mycelium developed from a spore sown in raw glue and gastric juice. The measurements were begun at 9.30 A.M. on the seventh day after sowing, the branch having just formed, and being then $6{\cdot}6\ \mu$ long.

Time.	Length.	Temperature.	Time.	Length.	Temperature.
	μ	° C.		μ	° C.
9.30 A.M.	6·6	23·0	11.50 A.M.	26·4	25·0
10.5 „	8·8	22·5	12.15 P.M.	31·9	26·0
10.45 „	14·3	23·5	12.35 „	37·4	26·5
11.15 „	22·0	24·5	2.0 „	77·0	26·0

Since, in this instance, the temperature was slowly rising most of the time, the following may be compared with it.

On a mycelium in a sister-cell, treated exactly like the foregoing, and of the same age, a side branch was fixed at 2.25 P.M. and then measured 92·4 μ. Its growth was as follows :—

Time.	Length.	Temperature.	Time.	Length.	Temperature.
	μ	° C.		μ	° C.
2.25 P.M.	92·4	24·5	7.20 P.M.	149·6	22·5
2.40 ,,	96·8	24·5	7.45 ,,	154·0	22·5
3.0 ,,	101·2	24·5	8.10 ,,	158·4	23·5
3.45 ,,	110·0	24·75	8.40 ,,	162·8	24·0
4.0 ,,	114·4	24·8	9.10 ,,	167·2	24·5
4.20 ,,	118·8	25·0	9.30 ,,	171·6	25·5
4.50 ,,	124·3	24·5	9.35 A.M.	281·6	23·0
5.10 ,,	129·8	24·25	2.0 P.M.	330·0	23·0
5.30 ,,	133·0	24·5	4.0 ,,	352·0	21·5
6.0 ,,	137·4	24·0	5.30 ,,	365·0	22·5

It must be remembered that in these cases the growth is not intercalary along the whole filament, but apical, *i.e.*, when a septum has been formed, the part behind ceases to elongate, and only the non-septate part in front goes on growing in length. It is necessary to bear this in mind when comparing the growth-curves of this fungus with those of a bacillus, such as *B. ramosus*.* The curve is similar in shape in both cases, but far less steep in the case of the fungus, and of course there is no point in reckoning the doubling periods in the latter.

The growth of *Onygena* seems to be relatively slow, but there is evidence to show that it quickens up as the drops become more diffluent and absorb water. This does not appear to be merely due to the temperature, but to a series of events in which, no doubt, freer access of oxygen, as well as increased digestive power on the part of the increasing mycelial surface, both play their parts.

So far the cultures only yielded such mycelia as could find space in the hanging drops, and these, of course, were soon exhausted. In the case of the most robust growths, I found a tendency to the rapid development of more or less tufted branches (figs. 38 and 39) towards the latter days of the growth, but this was the nearest approach to the formation of sporophores I was able to induce in these microscopic cultures. It was necessary to transfer the minute cultures to a larger supply of food material.

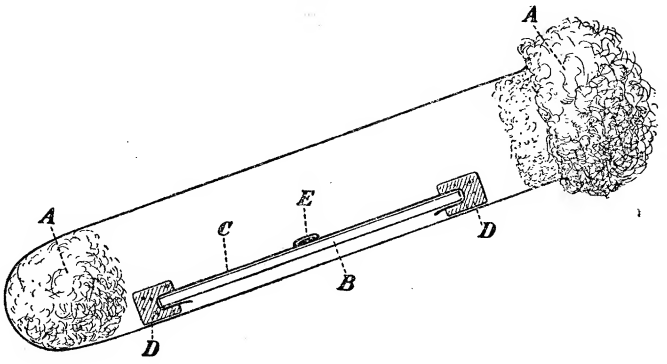
Mycelia, already well developed in glue solution and gastric juice, grew for a short time when transferred to slope tubes of glue-gelatine-broth, but the cultures were in most cases rapidly suppressed (at 16–18° C.) by overgrowing bacteria. Nor was I successful with similar transferences of the mycelia from glue and gastric juice to pieces of hoof, raw or boiled, and with or without gastric juice.

A number of attempts were made with the following arrangement :—A piece of thin horn shaving was stretched flat along a thin strip of glass, turned over its ends,

* See MARSHALL WARD, 'Proc. Roy. Soc.,' vol. 58, p. 38.

and clamped there by a piece of split cork. This culture strip was then put into a test-tube with cotton-wool below and the same as a plug, and sterilised with water (see fig. 1). Spores or transplanted mycelium could then be placed on the damp sterile horn strip (E in figure) with or without food materials, gastric juice, &c.

Fig. 1.



A = cotton wool. B = glass-slip. C = thin shaving of horn, clipped on to glass-slip at ends by D pieces of cork. E = sowing of fungus.

In several cases this method has already led to results which show that the horn can be directly attacked by the hyphæ, if previously invigorated by digestion.

Spores placed with a drop of hydrolised horn and gastric juice on such a shaving, on March 27, had germinated successfully by April 4, and put forth tubes 15–20 times as long as the spores.

Still more successful were experiments where I transferred strong mycelia, grown in hanging drops of suitable media. Thus, a mycelium a week old, grown from spores in raw glue and gastric juice, was transferred to the horn shaving on March 30, and a drop of cow-dung extract with gelatine and hydrolised horn added to start renewal of its growth. On April 4 the hyphæ had penetrated the horn, making their way in between the scales, and growing between them in an area as much as 8–10 millims. across.

Still more instructive was the following case:—Chlamydospores were sown in a hanging drop of coarse glue and gastric juice on March 24, and had formed a vigorous mycelium by March 30. This was transferred to a horn shaving on that day, and on April 4 had formed a patch 10–12 millims. in diameter, and some of the hyphæ had penetrated right through the horn shaving, as well as spread into its substance (fig. 41). Moreover, the superficial hyphæ had formed numerous chlamydospores (fig. 40), just as in the hanging drops described on p. 286.*

On comparing the results of the various experiments, it becomes more and more evident that the germination is faster, better, and leads to stronger mycelia the nearer the food material approaches the probable constitution of hydrolised horn, and every-

* During the summer two of these cultures have developed young sporophores on the horn shaving, attaining the stage shown in fig. 5, so that I have now the life-history complete on horn.—[Note added August 21, 1899.

thing seems to point to the products of such hydrolysis as the favourite food substances of the fungus. I therefore instituted a series of experiments to see if I could get any nearer to the bottom of the matter.

Very little appears to be known of the nature of horn. It contains a body known as *keratin*, which yields sulphuretted hydrogen on hydrolysis with water at high temperature, and it is dissolved on boiling with acids or alkalies, yielding leucin, tyrosin and some aspartic acids, with other as yet unknown products.

I began by dissolving horn chips in boiling dilute sulphuric acid. In this the horn rapidly softened, swelled and partially dissolved, while a penetrating, pungent odour escaped. The strongly acid liquor was then neutralised with barium carbonate, and when filtered yielded a bright yellow liquid, almost devoid of smell. This did not set on standing, but when a few drops were dried on glass, a tough membrane-like mass remained behind, easily soluble again in hot water.

I then proceeded on the assumption that this liquid might contain the nutrient food of the fungus—though, of course, it is as likely, or even more so, that it rather contains products such as the mycelium would itself cause to be formed as it extracted nutriment from the horn. Still there was the possibility that since I had stopped the hydrolysis long before all the horn was dissolved, the liquid contained in solution just those bodies which the mycelium normally seeks in the horn.

The results were not encouraging. Although some measure of success attended the sowings if gastric juice was added, the spores did not germinate nearly so well as in glue and gastric juice. Nevertheless, in some of the experiments with horn shavings, it appeared as if this medium was of use in starting the mycelia and rendering them sufficiently vigorous to penetrate the horn. The subject needs further investigation, and I hope to pursue it later.

It was found that if the outer parts of the decomposed cow's horn are shaved off with a sharp razor, leaving a white surface fully impregnated with the mycelium, the latter is still capable of giving rise to new sporophores in a damp atmosphere, and did so for many weeks, and some interesting new observations were obtained by watching the development of these under a bell-jar, lined with grey filter-paper kept damp and lifted daily to allow of the escape of the carbon dioxide and ammonia.

The sporophore appears first as a scarcely perceptible upheaval of snow-white mycelium, which bursts through between the decomposing scales of horn of the substratum. When it attains a height of about a millimetre, the dome-shaped mass assumes a peculiar glistening white appearance, as if powdered with fine snow; and closer observation shows that its surface is dusted over with loose cells: the peculiar character of which, and the air they entangle between them, render it extremely difficult to wet the structure, and obstruct the penetration of fixing fluids in which the organs are immersed. The loose cells behave almost as if they were greasy, and so obstinately is the air held between them, that even under a good pump it takes some time to sink the young sporophores.

Sections show that these dome-shaped incipient sporophores consist of densely interwoven, branched and sparsely septate, upgrowing hyphæ, the abundant contents of which are very rich in stainable materials (fig. 5).

Towards the upper parts of the domed apex, these hyphæ send numerous branches outwards—perpendicularly to the periphery of the dome—and as these approach the exterior they increase in thickness and their free ends swell and become more rapidly septate, resembling Indian clubs cut up into short segments.

The segments themselves then swell slightly into barrel-shaped portions, and some of them—usually the alternate segments—take up more of the hyphal contents than the others, swell up more and more, and obtain firmer walls (figs. 14–18). The starved segments which have given up their contents contract more and more, and eventually break, letting loose the barrel-shaped joints as separate spore-like cells, each showing remnants of the torn intermediate cells at the ends (figs. 16 and 17).

Further investigation showed that these are a hitherto undiscovered and second form of reproductive spore of *Onygena*.

It is these separated barrel-shaped—or, when free, egg-shaped—cells which form the white glistening powder on the young sporophores, and their development and structural relations alone suggested that they are *chlamydospores*. High powers and suitable staining show that each cell contains abundance of vacuolated protoplasm, in which a more deeply-coloured speck, apparently a nucleus, exists (figs. 16 and 17).

Although the mode of development, shape, and structure, and the separation of these cells point to their being acrogenously abstricted *chlamydospores*, like those of *Oligoporus*, *Nyctalis*, &c., with the sporogenous hypha compacted into a sort of stroma, no attempts to get them to germinate succeeded at first. I sowed them in all the ordinary media in vain: they seemed to share with the spores a resistance to germination which is only to be overcome by the action of special conditions.

Here, again, a clue was obtained by the success with the spores treated with gastric juice. They germinated readily in extract of cow-dung and gelatine to which gastric juice was added, as also in similarly treated solutions of raw glue, horn-extracts, &c., as already described for the ascospores. The process of germination always began by the emission of a germ-tube from the lateral wall, usually just below one end, and the hypha produced differed in no essential from the germinal hypha of the spores (*cf.* figs. 19, 20, and 21).

Further experience showed, however, that in certain solutions these chlamydospores are capable of germinating without the intervention of the gastric juice, though there could be no doubt that digestion promotes their germination considerably.

One reason for this may be that the chlamydospore, which is considerably larger than the ascospore (8–12 μ × 5–6 μ as against 6–7 μ × 4–5 μ), contains a much larger stock of protoplasm and reserve contents. As I have already entered into the question of the germination of the ascospores at some length, it will suffice to give the principal results with the chlamydospores in the following tabular form:—

Chlamydospores.

No. of experiment.	Composition of medium.	Treatment of chlamydospores.	Temperature.	Methods.	Period of experiment.	Results.	Remarks.
460	Glue, tapwater, Liebig-peptone . .	Fresh	23° C.	Cell	11 days	None germinated	
,,	,, ,, ,, . . .	,,	Room*	,,	,,	,,	* *i.e.*, the ordinary room-temperature: about 16–18° C.
,,	,, ,, ,, . . .	,,	23° C.	Tube	,,	,,	
,,	,, ,, ,, . . .	,,	Room	Plate	,,	,,	
472	Filtered and boiled dung-extract and gelatine and gastric juice	,,	23° C.	Cell	...	Germinated	
,,	Ditto ditto	,,	Room	,,	...	,,	
477	Glucose-amine and salts, gelatine and gastric juice	,,	23°	Cell	20 days	Negative	Two trials made.
,,	Ditto ditto	,,	,,	Tube	,,	,,	
483	Boiled coarse glue and gastric juice	,,	21° C.	Cell	8 days	Germinated	Two trials, and both developed splendid mycelia. In 2 days the tubes were 10–15 times length of chlamydospore.
,,	,, ,, ,, ,,	,,	,,	Tube	,,	,,	
484	Raw glue and gastric juice . . .	,,	,,	Cell	,,	,,	Two cells made. Germinated in 3 days; far behind 483, but good.
,,	,, ,, ,, ,, . . .	,,	,,	Tube	,,	,,	
485	,, ,, ,, ,, . .	Digested 24 hours in gastric juice at 20° C.	,,	Cell	,,	Negative	Two trials. Probably over digested.
,,	,, ,, ,, ,, . .		,,	Tube	,,	,,	
491	Hydrolised horn	Fresh	22° C.	Cell	7 days	Germinated	Two trials, both gave positive results. In 3 days developed tubes 10–20 times length of spore.
,,	,, ,, ...	,,	,,	Tube	,,	,,	
492	Hydrolised horn and gastric juice .	,,	,,	Cell	,,	Doubtful	All very foul with bacteria.
,,	,, ,, ,, ,, . .	,,	,,	Tube	,,	,,	
493	Digested coarse glue (boiled). . .	,,	,,	Cell	,,	Germinated	Two trials, both successful. In 3 days had formed tubes 2–5 times spore-length.
,,	,, ,, ,, . . .	,,	,,	Tube	,,	,,	
494	As last, but with gastric juice added	,,	,,	Cell	,,	,,	
,,	,, ,, ,, ,, .	,,	,,	,,	,,	,,	
498	Cow dung extract, gelatine, and hydrolised horn	Fresh, but soaked in water for 2 hours at 35° C.	23° C.	,,	,,	,,	Two experiments: one showed a few chlamydospores germinating on the fourth day.

In one or two cases I have been able to induce strong growths to form chlamydospores in the hanging drop. Thus, in sowings in coarse glue, boiled, to which gastric juice was added, the mycelia from the chlamydospores developed chlamydospores in chains on the eleventh day. We have already seen that similar developments occur on vigorous mycelia transferred to horn-shavings (p. 282, see fig. 40). I have not as yet succeeded in getting spores to do this in the hanging drops.

About the time when the *chlamydospores* have covered the whole upper portion of the stroma with a dense powder, the dome-shaped upper half of the fructification begins to swell and to form a globoid head (fig. 4) marked off from the pedicel below by its slightly increased diameter. Vertical sections through the whole structure at, or just prior to, this stage, show that while the chains of *chlamydospores* are becoming exhausted, a new set of structures—the ascogenous hyphæ—begins to form in the interior of the hyphal mass (fig. 4 and figs. 22–29).

Before describing the details of the changes now beginning in the interior, however, I may complete the history of the hyphal weft which, as just described, gave rise to the *chlamydospores*.

During the later stages, the hyphæ giving off the *chlamydospores* have become more and more densely compacted (fig. 14), and as the crop of *chlamydospores* ceases, are so crowded that their contiguous walls practically fuse by pressure and partial dissolution (fig. 18). It is these compacted broader hyphal ends, in which one or two further cell-divisions occur as a final act, which now constitute the *peridium* (fig. 7). The ragged torn ends on the exterior, and which give the roughness to the completed *peridium*, are nothing more than the remains of the separation-cells between the series of chlamydospores last formed; and, of course, the continuation of the inner cells of the peridium into the hyphal strands (*capillitium*) in the *gleba* is now intelligible (figs. 7 and 18).

I now return to the conditions just prior to the formation of this peridium.

Hitherto the general course of the branched and septate hyphæ has been upwards and outwards, like the stream of a fountain, and this upward course is still characteristic of the slender hyphæ forming the pedicel (fig. 5). In the mass forming the interior of the head, however, a number of dense knots of very short, closely septate, coral-like branches now make their appearance (fig. 5), each knot arising by branching from one of the upward hyphæ (fig. 22). The latter are, as already described, thin and sparsely septate, and soon lose most of their contents; but the short, knob-like branches forming these lateral knots are much thicker, and so densely filled with protoplasmic materials (figs. 23–27), which take up stains like hæmatoxylin in far greater quantity than any other hyphæ, that they stand forth sharply in the preparations (fig. 5). The short branches of each knot tend to curl over one towards another, reminding one of the fingers and thumb of an irregularly closed fist (to which, indeed, they are not inaptly compared if we suppose the wrist to represent the piece attaching the knot to the upright hyphæ), or they coil once or twice

(figs. 25*a* and 25*b*), and in some cases form dense knots (fig. 22). The horizontal outgrowth of each knot naturally forces the surrounding erect hyphæ aside, and as the knots are developed in large numbers, the impression is soon formed of a series of chambers, between sinuously up-growing hyphal strands, each chamber filled with the densely crowded and closely segmented coral-like knots, the pushing out of which has formed it.

These chambers are the chambers of the *gleba* (figs. 8 and 9), and the coral-like knots are the tufts of sporogenous hyphæ. When the latter have reached their full development, and have formed the spores, the hyphæ lining the chambers having given up all or most of their contents, and become pressed more and more closely together by the ascogenous hyphæ and spores, appear like plates or strands of empty hyphæ (capillitium) simply lining the chambers of the gleba, in which the ripe spores then lie loose (figs. 4 and 8).

The important question now arises, what intervenes between the inception of the tufts of sporogenous hyphæ and the maturation of the spores? Do the coral-like branches, richly supplied with protoplasm, develop into *asci*, or are the spores formed otherwise?

The presumption was—judging from TULASNE'S figures and from the descriptions referred to above (p. 270)—that the tufts are groups of ascogenous hyphæ. But I failed for some time to find any trace of ascus-formation at all; on the contrary, the spores appeared to be the direct result of segmentation of the short branches.

The method adopted to solve this difficult question was to cut serial microtome sections of the fructifications in every available stage of development, from the time when the tufts first appear, to that when the spores lie free and mature in the cavities of the gleba; and since, so far as I know, no one has hitherto traced this development, the details are of interest.

It is comparatively easy to obtain the earlier stages (*e.g.*, figs. 22 and 23), as already described, but those between the first swelling up of the segments to form asci (*e.g.*, fig. 25) and the fully-developed form (fig. 29) offer peculiar difficulties connected with the relative rapidity of formation, the coincidence of development of all the asci in the head, and the fugacious character of the ascus walls.

Soon after the inception of the ascogenous coils, the short, barrel-shaped segments (fig. 24) begin to show signs of differentiation of their contents, vacuoles appear, and in some cases a nucleus can be observed (figs. 26, 27, and 28 *a* and *b*). A comparison of numerous preparations shows that the stage with one nucleus is soon followed by one where two co-exist (fig. 28*c*). Then asci with four, and finally with eight nuclei are found (fig. 28 *d* and *e*), and in slightly more advanced heads the fully formed asci, each with eight spores, may be found (fig. 29).

These rapidly burst, and let the ripening spores escape into the meshes between the capillitium (figs. 4, 8, 9, and 10), and the gleba now appears as a powdery snuff-

like mass of the spores (fig. 2 *c*). Each young ascus in the stage shown in fig. 29 measures about 10 μ in diameter.

No trace of any process comparable with the sexual process in Erysipheæ, or the fusions in *Eurotium*, &c., could be discovered at any stage in the development.

Reviewing the whole course of development of these sporophores of *Onygena*, an interesting point may be suggested bearing on the often quoted parallelism between members of the two great groups of Fungi—the Ascomycetes and the Basidiomycetes—*e.g.*, between Tuberaceæ and Gastromycetes, between *Exoascus* and *Exobasidium*, &c.

If we examine BREFELD'S masterly exposition of the structure and development of *Pilacre*, a member of the Protobasidiomycetes, it will be noticed that in habit—not in habitat, however—size, and other external characters, as well as in general structure, there are decided resemblances to *Onygena*. Moreover, *Pilacre* begins by forming a clavate mass of up-growing hyphæ, the outer ends of which first develop *conidia* and then mat together to form a *peridium*; much in the same way as in *Onygena* they form *chlamydospores* and then mat together to produce the peridium. This done, in both cases the sporogenous branches develop in tufts from the hyphæ below the peridium, and although in *Pilacre* these become basidia with basidiospores, whereas in *Onygena* they develop into asci containing ascospores, their arrangement, mode of origin, and general course of development are strikingly analogous in the two cases.

I cannot help thinking that just as *Pilacre* may be looked upon as a primitive form leading to the Gastromycetes through such genera as *Tulostoma*, so the foregoing results show that *Onygena* might be regarded as leading to the Truffles if a connecting pedicillate form of the *Balsamea* type were known. It may be noted that the arrangement of the "capillitium" fibres is strikingly similar to that of the septa in Tuberaceæ.

It is interesting to note—though the significance is probably historical only—that previous observers, knowing nothing of the development or even of the asci, had already described a species of *Pilacre** as an *Onygena*, misled no doubt by the resemblances referred to above.

Although much remains to be done before the questions raised are all solved, it seems probable that the main facts of the life-history of this curious fungus are now elucidated. We may infer that in nature it attacks the horn, hair, hoofs, feathers, &c., of animals and birds, and that the spores are licked off or swallowed as the feathers are preened. During passage through the alimentary canal, the action of the gastric juices favours germination, which probably occurs in the dung. The germinated mycelium probably then reaches the horns, hoofs, feathers, &c., again, in various ways —*e.g.*, by the animal treading in the dung, or lying down on soiled ground, and so forth—and so the cycle is renewed. Possibly the reason *Onygena* is so rarely seen on

* See COOKE, 'Handbook of British Fungi,' vol. 2, p. 625.

the living animal—it does sometimes occur there*—is that so long as the animal is vigorous, it cleans itself efficiently.

Explanation of Plates.

PLATE 21.

Fig. 1. *Onygena equina* on a cow's horn, showing fructifications (sporocarps). Natural size.†

Fig. 2. Isolated sporocarps and groups of the same: *a* and *b*, natural size; *c*, slightly magnified, and showing the rupturing peridium.

Fig. 3. Vertical section through a mature sporocarp under a low power.

Fig. 4. Similar section through upper part of a nearly ripe sporocarp, showing spores in the meshes of the *gleba*, *peridium*, *sub-hymenial layer*, and pedicel. Zeiss B, occ. 4.

Fig. 5. Similar section through a much younger sporocarp before the peridium, spores, and sub-hymenial layer have been developed. The head is covered with *chlamydospores*, and the dark patches in the young *gleba* are the tufts of ascogenous hyphæ. Zeiss C, occ. 4.

Fig. 6. Small portion of a transverse section of lower part of pedicel, showing transversely cut hyphæ, and three scales of horn also in section. The two dark bodies are young spores accidentally washed into the preparation. Zeiss D, occ. 4.

Fig. 7. Portion of a vertical section of the peridium of a ripe sporocarp, showing the gradual passage of the hyphæ—*capillitium*—of the gleba into pseudo-parenchyma, the outer cells of which are torn and jagged. Apochrom. 0·4 millim., occ. 4.

Fig. 8. Portion of peridium and meshwork of gleba with spores. Zeiss C, occ. 4.

Fig. 9. Part of gleba with spores in the chamber-like meshes. Zeiss C, occ. 4.

Fig. 10. Vertical section through sub-hymenial layer, showing how its closely compacted tissue passes above into the gleba and below into the looser hyphæ of the pedicel. Apo. 0·4, occ. 2.

Fig. 11. Mycelium in the horny substratum. From a thin section cut tangentially to the surface; the shaded patches are plates of decomposing horn. Zeiss D, occ. 2.

Fig. 12. Similar preparation, showing hyphæ free from the horn, and the deeply-stained granules in the protoplasm. Zeiss D, occ. 2.

* See Fischer, *loc. cit.*, p. 106.

† This figure, and figs. 2–6 and 8–12, were drawn for me by Miss Dale, to whom I here record my thanks.

PLATE 22.

Fig. 13. Part of latter more highly magnified, showing a septum, branch, anastomosis, and the deeply-stained swollen portions. Immersion, $\frac{1}{12}$.

Fig. 14. Portion of outer surface of a head in the stage shown in fig. 5, in vertical section, showing the series of chlamydospores. Apo. 0·4, occ. 4.

Fig. 15. Similar preparation in potassium hydrate, and more highly magnified. Oil immersion, $\frac{1}{12}$, occ. 4.

Fig. 16. Chlamydospores stained with iodine-green and fuchsin, and showing the nuclei. Apo. 0·4.

Fig. 17. Chlamydospores. The upper ones stained with hæmatoxylin, and showing nuclei and vacuolisation. Oil immersion, $\frac{1}{12}$.

Fig. 18. Vertical section of outer parts of a sporophore, somewhat older than fig. 5, but younger than fig. 4. The crops of chlamydospores are now coming to an end, and the hyphæ which formed them are becoming compacted into the pseudo-parenchyma of the peridium (*cf.* the later stage in fig. 7). Internally, these hyphæ pass into those of the *gleba*, in which young spores are already visible. Apo. 0·4, occ. 4.

Fig. 19. Chlamydospores germinating in boiled coarse glue + gastric juice at 20° C.–22° C., after about 48 hours. Zeiss D, occ. 4.

Fig. 20. The specimen marked *a* in last figure traced further : *a*, on March 26, *t.* = 21° C. ; *b*, on March 27, *t.* = 18° C. ; *c*, on March 28, *t.* = 21° C. ; *d*, on March 29, *t.* = 20° C. ; *e*, on March 30, *t.* = 21° C. The figures *a* to *d*, under Zeiss D, occ. 4 ; *e*, under Zeiss A, occ. 2.

PLATE 23.

Fig. 21. A group of chlamydospores, germinating in cow-dung, gelatine, and gastric juice, on the third day after sowing. Immersion, $\frac{1}{12}$.

Fig. 22. Groups of young ascogenous tufts of hyphæ, dissected out by pressure from section of sporophores in the stage shown in fig. 5, treated with eau de Javelle and glycerine. Immersion, $\frac{1}{12}$.

Fig. 23. Similar groups from stained preparations in balsam, showing the dense contents of the ascogenous hyphæ. Immersion, $\frac{1}{12}$.

Fig. 24. Two similar tufts, slightly older, showing the swelling of the segments. Immersion, $\frac{1}{12}$.

Fig. 25. A still more advanced stage : the segments are now rapidly swelling up into asci. Immersion, $\frac{1}{12}$.

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Fig. 29. A group of three nearly ripe asci, the upper one with eight spores. Immersion, $\frac{1}{12}$.

Fig. 30. Half-a-dozen ripe ascospores as they lie in the chambers of the gleba (*cf.* figs. 3 and 4), each showing two oily drops. Zeiss D, occ. 4.

Fig. 31. Nearly ripe spores, stained with hæmatoxylin, and showing nucleus-like masses in the protoplasm. Apo. 0·4, occ. 4.

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PLATE 24.

Fig. 35. Abnormal germination of ascospores in glucose-amine-gelatine and minerals, after treatment with gastric juice, at 23° C. After 10 days, the spore had germinated (*a*) and formed a curious vesicle, as if about to produce another spore, and half the germinal tube had become empty, except for a few granules, a septum dividing the two moieties. *b* shows the condition 24 hours later; *c*, 48 hours later; *d*, 48 hours later still, and *e* after yet another 48 hours. It never became a healthy mycelium.

Fig. 36. Mycelium after 5 days' germination in glue + gastric juice at 23° C. Zeiss D, occ. 4.

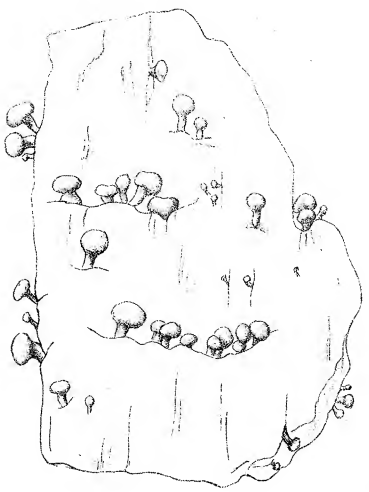
Fig. 37. Similar growth in cow-dung gelatine + gastric juice, on the 7th day, at 23° C.

Fig. 38. Crowded branchlets, on a 14 days' culture, as last. Zeiss C, occ. 4.

Fig. 39. Portion of last preparation more highly magnified. Zeiss E, occ. 4.

Fig. 40. Chlamydospores formed on a mycelium with which a horn shaving was infected (see p. 282). Zeiss D, occ. 4.

Fig. 41. A piece of horn shaving artificially infected. The hyphæ have penetrated the horn, and a branch has emerged to the exterior (see p. 282). Zeiss D, occ. 4.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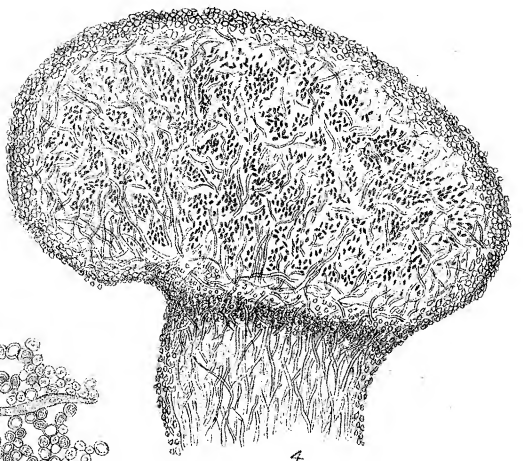
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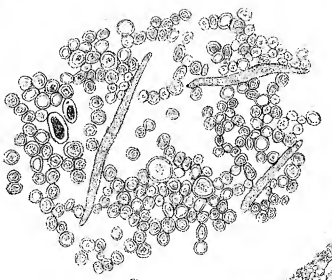
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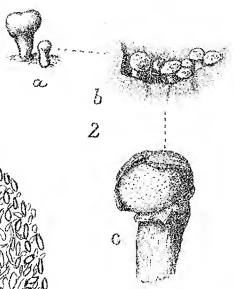
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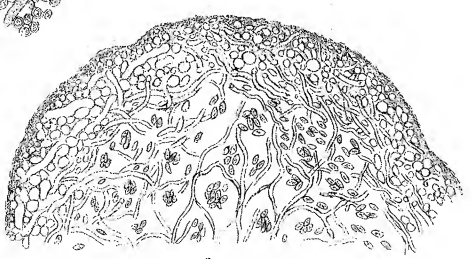
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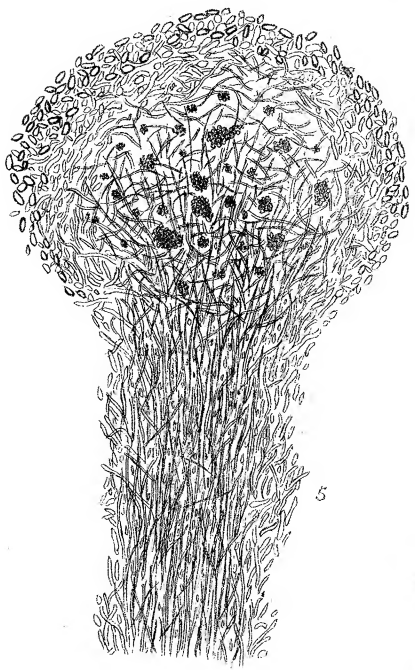
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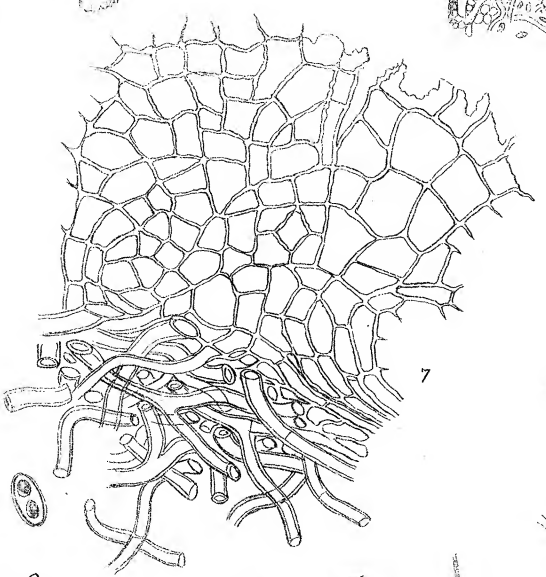
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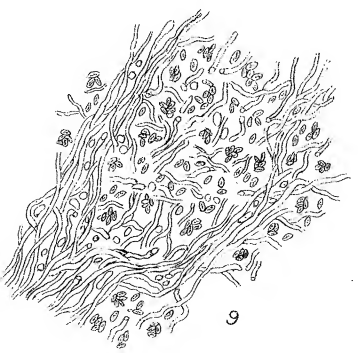
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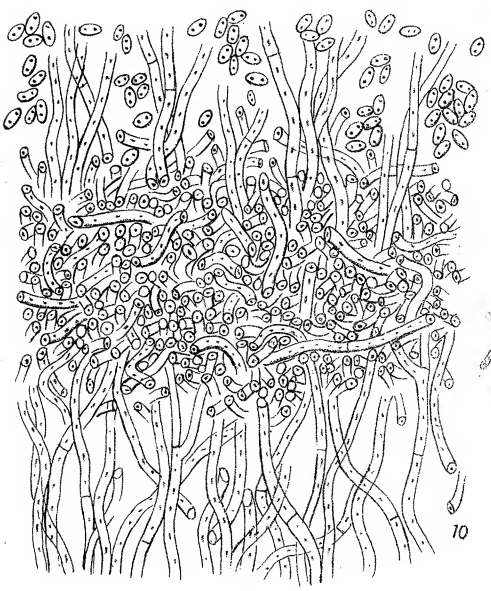
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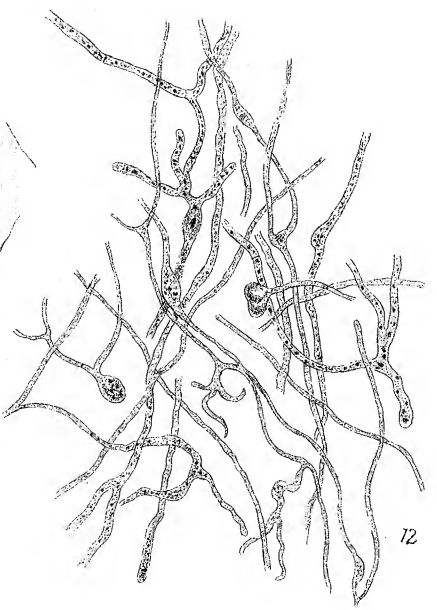
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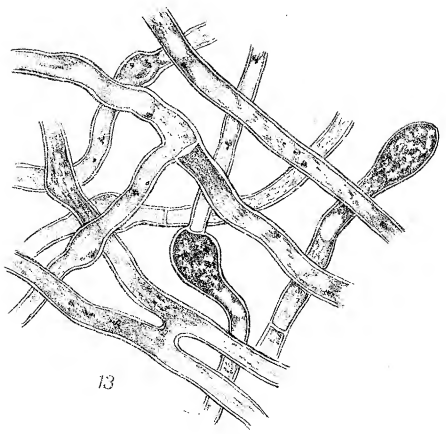
ONYGENA.

P. Highley lith.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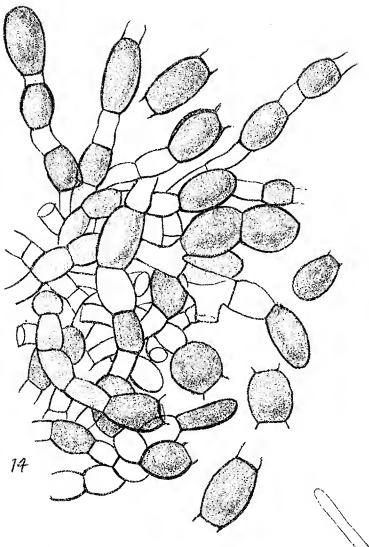
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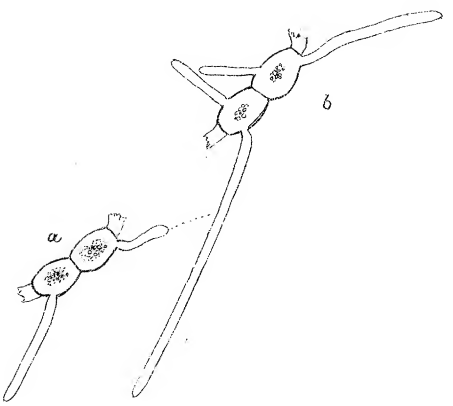

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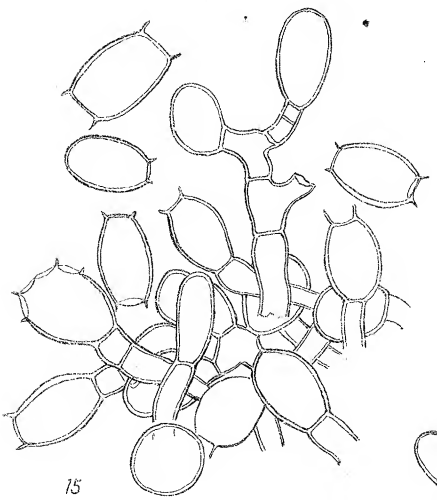
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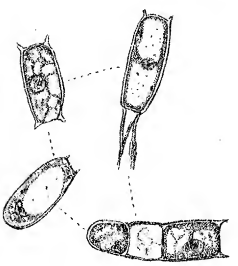

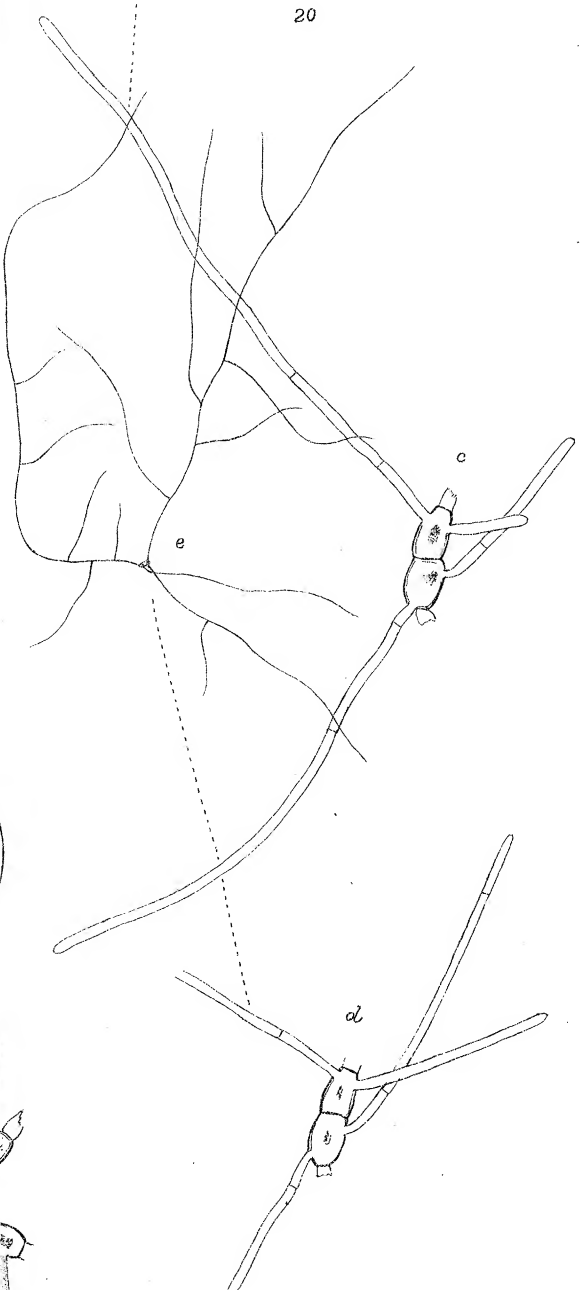

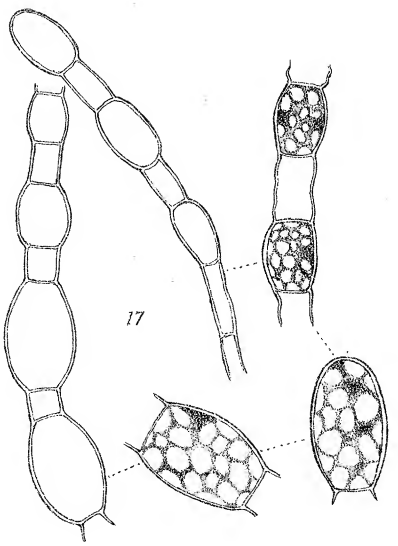
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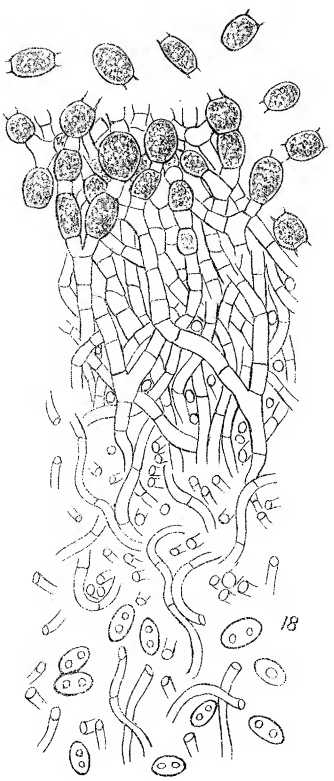
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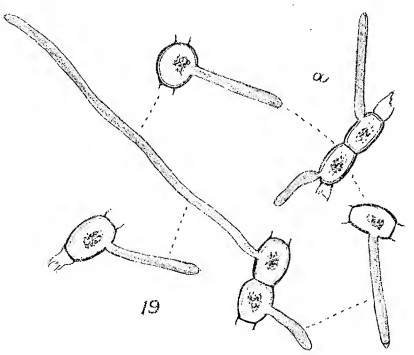

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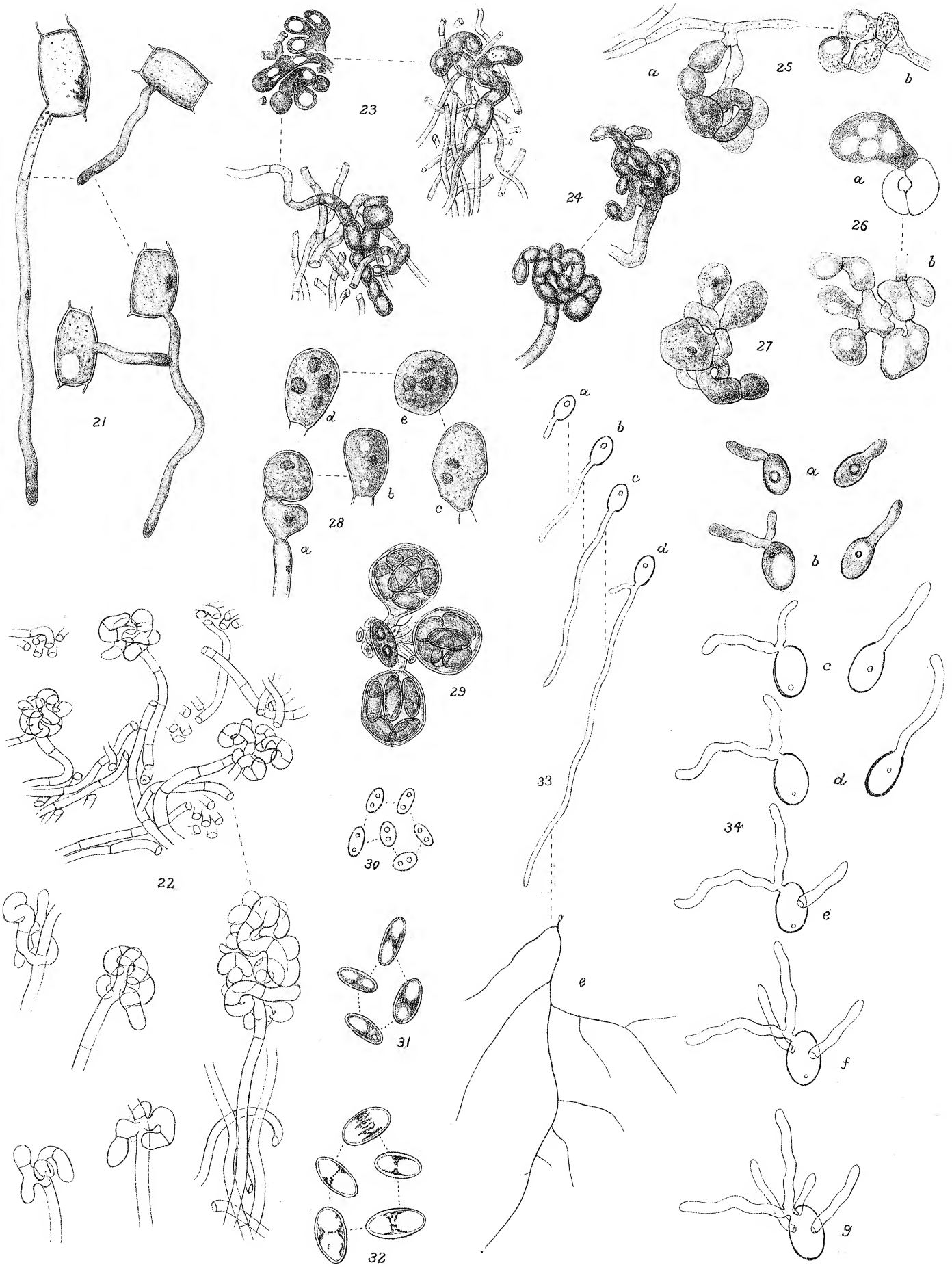

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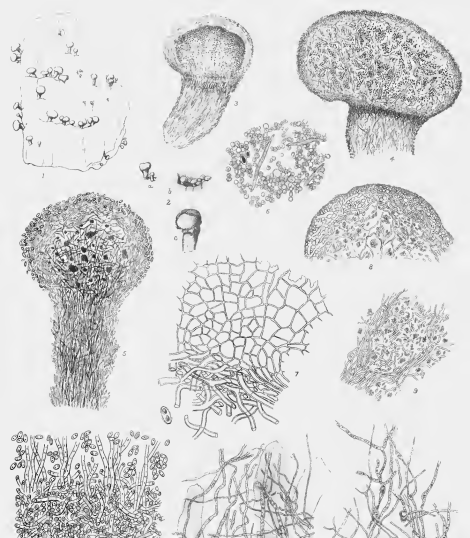

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PLATE 21.

Fig. 1. *Onygena equina* on a cow's horn, showing fructifications (sporocarps). Natural size.†

Fig. 2. Isolated sporocarps and groups of the same: *a* and *b*, natural size; *c*, slightly magnified, and showing the rupturing peridium.

Fig. 3. Vertical section through a mature sporocarp under a low power.

Fig. 4. Similar section through upper part of a nearly ripe sporocarp, showing spores in the meshes of the *gleba*, *peridium*, *sub-hymenial layer*, and pedicel. Zeiss B, oc. 4.

Fig. 5. Similar section through a much younger sporocarp before the peridium, spores, and sub-hymenial layer have been developed. The head is covered with *chlamydospores*, and the dark patches in the young *gleba* are the tufts of ascogenous hyphæ. Zeiss C, oc. 4.

Fig. 6. Small portion of a transverse section of lower part of pedicel, showing transversely cut hyphæ, and three scales of horn also in section. The two dark bodies are young spores accidentally washed into the preparation. Zeiss D, oc. 4.

Fig. 7. Portion of a vertical section of the peridium of a ripe sporocarp, showing the gradual passage of the hyphæ—*capillitium*—of the gleba into pseudo-parenchyma, the outer cells of which are torn and jagged. Apochrom. 0·4 millim., oc. 4.

Fig. 8. Portion of peridium and meshwork of gleba with spores. Zeiss C, oc. 4.

Fig. 9. Part of gleba with spores in the chamber-like meshes. Zeiss C, oc. 4.

Fig. 10. Vertical section through sub-hymenial layer, showing how its closely compacted tissue passes above into the gleba and below into the looser hyphæ of the pedicel. Apo. 0·4, oc. 2.

Fig. 11. Mycelium in the horny substratum. From a thin section cut tangentially to the surface; the shaded patches are plates of decomposing horn. Zeiss D, oc. 2.

Fig. 12. Similar preparation, showing hyphæ free from the horn, and the deeply-stained granules in the protoplasm. Zeiss D, oc. 2.

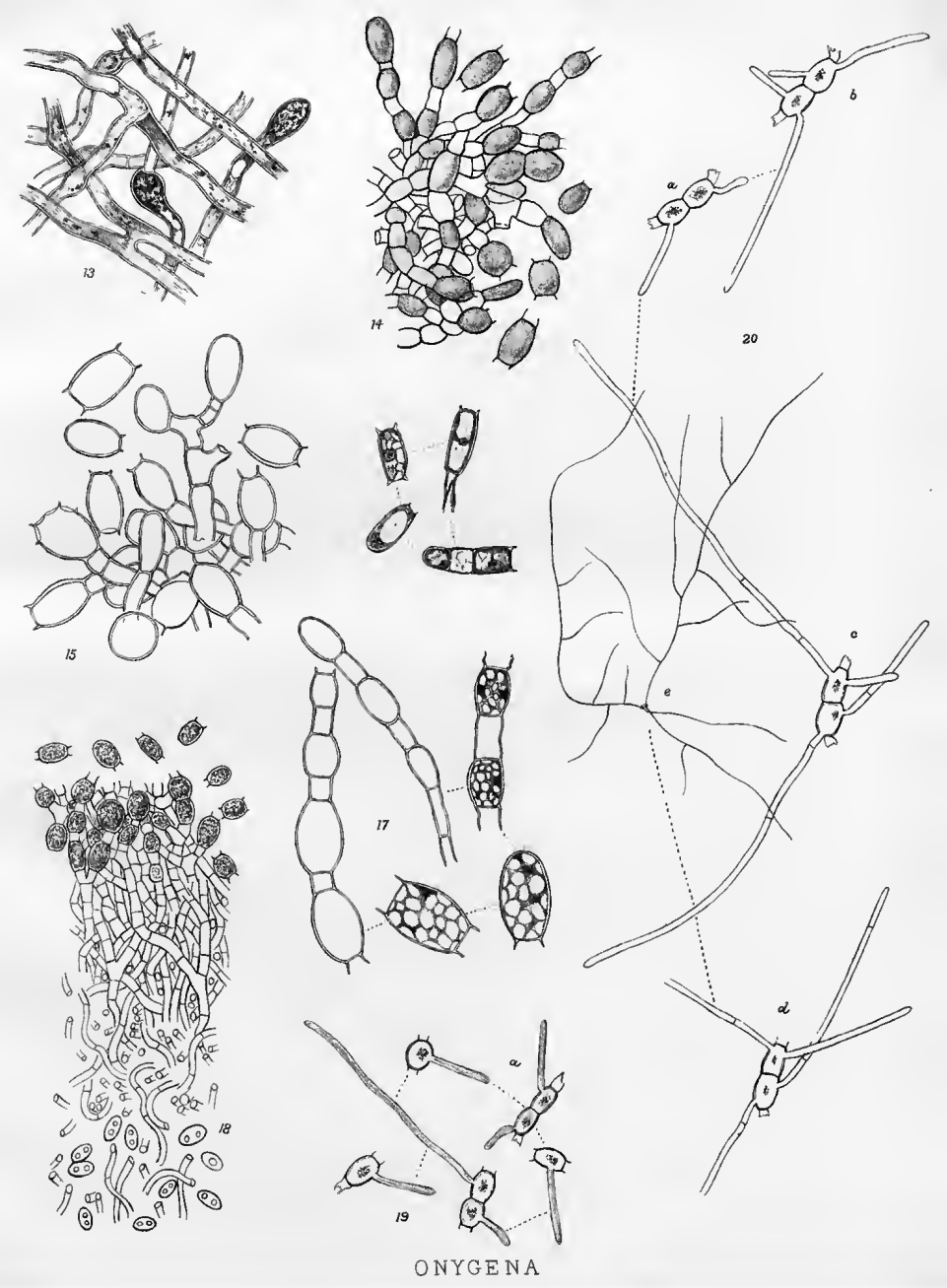

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PLATE 22.

Fig. 13. Part of latter more highly magnified, showing a septum, branch, anastomosis, and the deeply-stained swollen portions. Immersion, $\frac{1}{12}$.

Fig. 14. Portion of outer surface of a head in the stage shown in fig. 5, in vertical section, showing the series of chlamydospores. Apo. 0·4, occ. 4.

Fig. 15. Similar preparation in potassium hydrate, and more highly magnified. Oil immersion, $\frac{1}{12}$, occ. 4.

Fig. 16. Chlamydospores stained with iodine-green and fuchsin, and showing the nuclei. Apo. 0·4.

Fig. 17. Chlamydospores. The upper ones stained with hæmatoxylin, and showing nuclei and vacuolisation. Oil immersion, $\frac{1}{12}$.

Fig. 18. Vertical section of outer parts of a sporophore, somewhat older than fig. 5, but younger than fig. 4. The crops of chlamydospores are now coming to an end, and the hyphæ which formed them are becoming compacted into the pseudo-parenchyma of the peridium (*cf.* the later stage in fig. 7). Internally, these hyphæ pass into those of the *gleba*, in which young spores are already visible. Apo. 0·4, occ. 4.

Fig. 19. Chlamydospores germinating in boiled coarse glue + gastric juice at 20° C.–22° C., after about 48 hours. Zeiss D, occ. 4.

Fig. 20. The specimen marked *a* in last figure traced further : *a*, on March 26, *t.* = 21° C. ; *b*, on March 27, *t.* = 18° C. ; *c*, on March 28, *t.* = 21° C. ; *d*, on March 29, *t.* = 20° C. ; *e*, on March 30, *t.* = 21° C. The figures *a* to *d*, under Zeiss D, occ. 4 ; *e*, under Zeiss A, occ. 2.

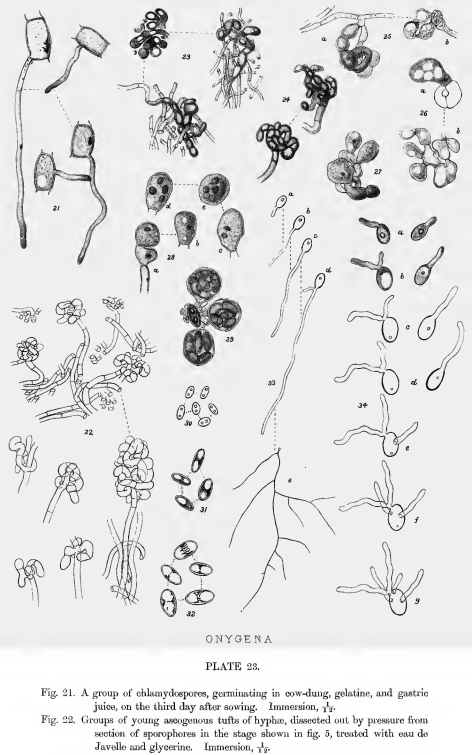

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PLATE 23.

Fig. 21. A group of chlamydospores, germinating in cow-dung, gelatine, and gastric juice, on the third day after sowing. Immersion, $\frac{1}{12}$.

Fig. 22. Groups of young ascogenous tufts of hyphæ, dissected out by pressure from section of sporophores in the stage shown in fig. 5, treated with eau de Javelle and glycerine. Immersion, $\frac{1}{12}$.

Fig. 23. Similar groups from stained preparations in balsam, showing the dense contents of the ascogenous hyphæ. Immersion, $\frac{1}{12}$.

Fig. 24. Two similar tufts, slightly older, showing the swelling of the segments. Immersion, $\frac{1}{12}$.

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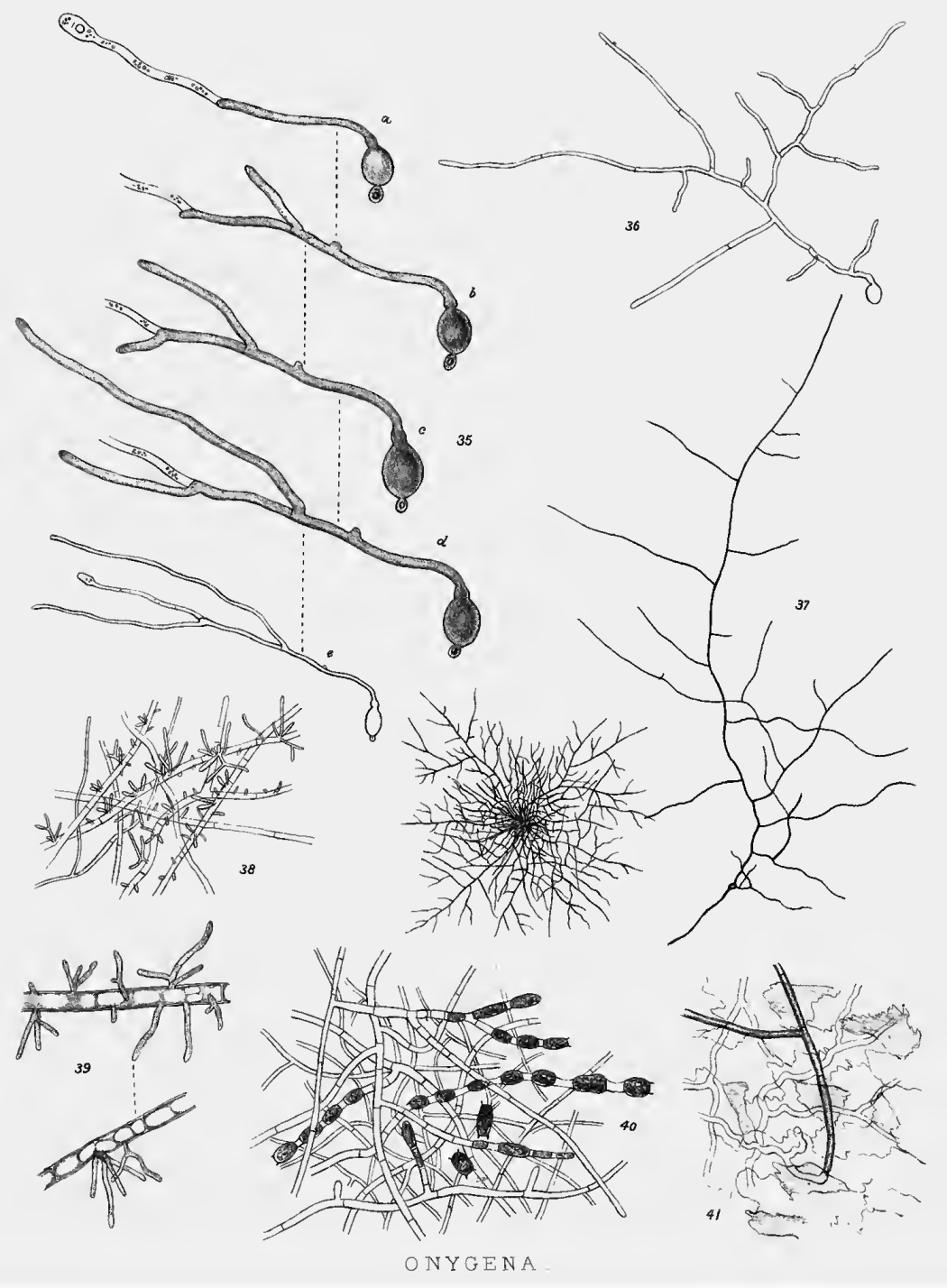

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